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(54) Title: CEREBROVASCULAR AMYLOID PROTEIN-SPECIFIC MONOCLONAL ANTIBODY SV17-6E10

(57) Abstract

Disclosed is monoclonal antibody SV17-6E10 and a specific-binding fragment thereof which is specifically reactive with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin. Also disclosed is a hybridoma cell line capable of producing the monoclonal antibody, a reagent composition which incorporates the monoclonal antibody or specific-binding fragments thereof and an immunoassay method for their use.

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Cerebrovascular amyloid protein-specific monoclonal antibody SV17-6E10

BACKGROUND OF THE INVENTION

Field Of The Invention

5   The present invention relates to
neurological disorders, and, more particularly, to
antibodies that are specific for peptides associated
therewith.

Brief Description Of The Prior Art

10   Alzheimer's disease is characterized by
three major pathological markers. They are neuritic
plaques, neurofibrillary tangles and cerebrovascular
amyloidosis. Glenner, et al., Biochem. Biophys. Res.
Comm., 120:885 (1984) and Wisniewski, et al., Int.

15   Symp. Dementia and Amyloid "Neuropathology", Suppl.
3:87 (1986). The accumulation of two types of
aberrant fibrils, the paired helical filaments and
the amyloid are characterized neuropathologically in

20   Alzheimer's disease/senile dementia of the Alzheimer
type. Plaque and cerebrovascular amyloid are
primarily made up of a 40- to 42- amino acid residue
peptide, called the B-peptide.

          Alzheimer's cerebrovascular amyloid protein

25   has been purified and a 24 amino acid residue
sequence has been reported. Glenner, et al., Biochem.
Biophys. Res. Comm., 122:1131 (1984). It has also
been observed that the sequenced portion of the
cerebrovascular amyloid peptide is part of the

30   sequence of a precursor protein. Robakis, et al.,
Proc. Nat. Acad. Sci. USA, 84:4190 (1987) and
Robakis, et al., The Lancet, 1:384 (1987).

          Down's Syndrome is a disability characterized
by the inheritance of an extra copy of chromosome 21

35   in each cell. Older persons afflicted with Down's

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Syndrome display dementia that resembles Alzheimer's
disease. The cerebral tissues of these individuals
exhibit the same neuropathological findings of
Alzheimer's disease, i.e., amyloid-containing
neuritic plaques, neurofibrillary tangles and
cerebrovascular amyloidosis. The amyloid deposits of
Down's Syndrome contain the same peptide as the
amyloid deposits found in the brains of Alzheimer's
disease victims. See, Glenner et al., Biochem.
Biophys. Res. Comm., 122:1131 (1984).

As applied to other peptides and antigens,
including many associated with various diseases,
monoclonal antibody products of hybridoma technology
has often proven valuable in studying factors
associated with these diseases and in diagnosis of
the disease in clinical settings. Kim, et al.,
Neuroscience Research Communications, 2:121 (1988)
discloses production and characterization of
monoclonal antibodies specific for the synthetic
cerebrovascular amyloid protein described in Glenner
et al., Biochem. Biophys. Res. Comm., 122:1131
(1984), supra.

SUMMARY OF THE INVENTION

Hybridoma technology is one of the most
important biological tools for the analysis of complex
antigens. Using the SV17-6E10 monoclonal antibodies
of the present invention, which are specific to a
recently acquired amino acid sequence of the
beta-amyloid precursor protein, it has become possible
to study the beta-amyloid precursor protein which
leads to significant deposits of vascular and neuritic
amyloid plaques in Alzheimer's disease and Down's
syndrome. These monoclonal antibodies provide the
potential for non-invasive diagnosis of Alzheimer's

disease using body fluids such as spinal fluid, serum
or urine.

   In one aspect, the invention provides a
monoclonal antibody, designated SV17-6E10, and
antigen - binding fragments thereof which are
specifically reactive with a peptide whose
concentration level is elevated in individuals having
Alzheimer's disease or Down's syndrome as compared to
individuals of substantially the same age who are not
so-afflicted and which does not react with other
peptides of human origin. The monoclonal antibodies
of the invention are of subclass IgG_1.
Papain-digestion produced antigen-binding fragments
(e.g. Fab) have also been shown to specifically
recognize amyloid plaque.

   The monoclonal antibody is produced by a
hybridoma formed by fusion of cells from a myeloma
line, usually of mouse origin, and antibody-producing
cells, also usually of mouse origin, previously
immunized with a peptide whose concentration level is
elevated in individuals having Alzheimer's disease or
Down's syndrome as compared to individuals of
substantially the same age who are not so-afflicted
and which do not react with other peptides of human
origin, such as one which forms a portion of a
cerebrovascular amyloid protein characteristic of
Alzheimer's disease or Down's syndrome. Preferably,
a mouse NSO myeloma line is used, particularly with
antibody-producing cells from a mouse previously
immunized with a peptide comprising the sequence
comprising asp - ala - glu - phe - arg - his - asp -
ser - gly - tyr - glu - val - his - his - gln - lys -
leu. Further in this regard, the invention provides
a hybridoma cell line capable of producing the
monoclonal antibodies described above.

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In another aspect, the invention provides a composition for quantitatively determining a peptide whose concentration level characteristic of Alzheimer's disease or Down's syndrome in an individual. The composition comprises a monoclonal antibody or antigen - binding fragment thereof in accordance with the invention and a detectable moiety which is directly or indirectly associated therewith. In one embodiment, the monoclonal antibody is conjugated to an enzyme and the detectable moiety comprises a chromogenic redox substrate for the enzyme. In another embodiment, the monoclonal antibody is conjugated to one partner of a specific binding pair and the other partner of the specific binding pair is conjugated to a detectable moiety or a substance capable of rendering the moiety detectable. One example of this embodiment is where the monoclonal antibody or antigen - binding fragment thereof additionally serves as an antigen of the specific binding pair. The partner for the monoclonal or fragment is an anti-immunoglobulin antibody, usually anti - IgG and usually from a species other than that of its partner, which is labeled, such as with a fluorophore like fluorescein isothiocyanate. Another example is where one partner of the specific binding pair is selected from biotin and its binding analogs and the other partner is selected from avidin and its binding analogs. Usually, the detectable moiety is a chromophore, fluorophore or luminophore and the substance capable of rendering it detectable is an energy donor or catalyst therefor.

Further in this regard, the invention provides an immunoassay method. The method comprises contacting a sample, from the individual suspected of having Alzheimer's disease or Down's syndrome, with

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the composition of the invention and quantitatively observing any detectable response.

Monoclonal antibody SV17-6E10 is very unique in that no monoclonal antibody reactive to this peptide (17 amino acid residue) has previously been reported. Monoclonal antibody SV17-6E10 has been used, as exemplified below, in immunoassays for characterizing the amyloid peptide and precursor amyloid peptides in connection with biogenesis of B-amyloid peptide plaques found in Alzheimer's disease and Down's syndrome afflicted brains.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1(a) shows amyloid plaques stained with SV17-6E10 monoclonal antibody.

Fig. 1(b) shows a serial section stained with SCVAP-2F9 monoclonal antibody. A serial section stained with SCVAP-4G8 monoclonal antibody produced results identical to those shown in Fig. 1(b).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1
Monoclonal Antibody SV17-6E10 to Amyloid Peptide

In the experiments reported by this example, a synthetic peptide, an antigenic conjugate thereof and tryptic fragments of the peptide were used to prepare and characterize the monoclonal antibody of the present invention.

17 Amino Acid Residue Synthetic B-Amyloid Peptide (SV17)

Preparation of SV17 was as follows. A 17-residue synthetic cerebrovascular amyloid peptide (SV17), having the sequence asp - ala - glu - phe - arg - his - asp - ser - gly - tyr - gln - val - his -

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his - glu - lys - leu, corresponding to a portion of
the published sequence of the Alzheimer's
cerebrovascular amyloid peptide was obtained from
Biosearch Laboratories, San Rafael, CA. The sequence
mentioned was published in Glenner, et al., Biochem.
Biophys. Res. Comm., 120:885 (1984). The preparation
as received was purified by reverse phase liquid
chromatography. The purified SV17 peptide (1 mg) was
conjugated to keyhole-limpet hemocyanin (6 mg) with
glutaraldehyde (3 ul) in sodium phosphate (1.2 ml, 50
mM, pH 7.5) at 4°C for 2 hours to provide the
SV17-KLH antigen.

Preparation of Hybrid Cell Lines

Procedures for producing antibody-secreting
hybrid cell lines as described in Togashi, et al.,
Arch. Virol., 67:149 (1981) and modified as described
in Kim, et al., J. Clin. Microbiol., 18:331 (1983)
were applied as follows.

BALB/CJ female mice (Jackson Laboratories,
Bar Harbor, ME) were immunized via the back foot pads
with Ribi adjuvant (200 ul) (Ribi Immunochem
Research, Inc., Hamilton, MT) containing SV17-KLH
antigen (25-50 ug). At 21 days after the initial
injection, these mice were immunized
intraperitoneally with SV17-KLH antigen (25-50 ug) in
Ribi adjuvant (200 ul) four times at about four week
intervals. Four days prior to fusion (at six weeks
after the last immunization) the mice received
intraperitoneal booster injections of SV17 (200 ug)
without adjuvant. Immune spleen cells were harvested
as described in Galfre, et al., Methods Enzymol.,
3:73 (1981). A mouse with the highest titer after
SV17-KLH antigen immunization was selected for hybrid
clone production.

NSO myeloma cells were obtained from Dr.
Choi, Kyung Hee of Albert Einstein Medical School,
New York, NY.   The immune spleen cells were incubated
with the NSO myeloma cells and fused at a ratio of
5:1 in the presence of polyethyleneglycol 1500
(Boehringer Mannheim, Indianapolis, IN) as the fusing
agent.   The cells were maintained in Iscoves modified
Dulbecco minimum essential medium containing 15%
fetal bovine serum, 10^{-4} M hypoxanthine, 1.6 x
10^{-5} M thymidine and 4.0 x 10^{-7} M aminopterin for
14 days.   Subsequent feedings with medium containing
only hypoxanthine and thymidine were performed on
days 18, 22 and 25. After day 25, cells were fed with
medium that did not include hypoxanthine, thymidine
or aminopterin.   Normal BALB/CJ mouse macrophages
(6,000 cells per well in 96 wells of a microtiter
plate) as a feeder layer were used for the initial
hybridoma production and also for subcloning the
selected hybrids by limiting dilutions.

Establishment of Hybrid Clones Secreting Anti-SV17
mABs

After the initial hybridoma production, as
described above, 5 x 10^4 hybridoma cells in 1.0 ml
were added to each well of 96 well tissue culture
plates.   Hybrid cell growth was detected by the ELISA
technique using the synthetic peptide antigen (1
ug/ml) coated on the wells.   Supernatant fluid in
several wells were found to contain SV17 - specific
antibody.

The hybrid that produced SV17-6E10 antibody,
as determined by the ELISA procedure, was established
and cloned three times by limiting dilutions to
obtain a pure clone.   Normal BALB/CJ mouse
macrophages (6,000 cells per well in 96 wells of a
microtiter plate) as a feeder layer were used here

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also. Stable cloned hybrid cell lines were
established and are referred to here as purified
antibodies.

Example 2
Monoclonal Antibody SV17-6E10
Immunocytochemistry Reagent

Monoclonal antibody SV17-6E10 was used in
the experiments reported by this example as an
immunocytochemistry reagent. Paraffin -embedded,
formalin - fixed Alzheimer and normal control cortex
sections (6 um) were stained using the avidin-biotin
complex technique (Bethesda Research Laboratories,
Bethesda, MD). Amyloid present in neuritic plaques
and cortical and meningeal vessels in brain sections
of Alzheimer's patients were stained and those of
non-afflicted controls were not. No staining of
Alzheimer neurofibrillary tangles or
axons/neurofilaments either in tissue sections or in
isolated neurons were observed with monoclonal
antibody SV17-6E10.

The immunoreactivity of monoclonal antibody
SV17-6E10 was then compared with that of SVCAP-2F9
(or SVCAP-4G8) on the amyloid plaques. Comparison of
their immunoreactivities with amyloid plaques using
serial sections (6 um thickness) revealed that there
were two types of amyloid plaques present.
Regardless of high concentration of SV17-6E10 used,
one group of plaques was lightly stained while the
other group of plaques was darkly stained (Fig. 1a).
When SCVAP-2F9 or SCVAP-4G8 monoclonal antibody was
used, these two groups of plaques all reacted with
equal staining intensity (Fig. 1b). Monoclonal
antibody SV17-6E10 was raised against the amino acid
residue 1-17 of the synthetic amyloid B-protein while
SCVAP-2F9 and SCVAP-4G8 are monoclonal antibodies

specific to an epitope in the 17-24 amino acid
segment of the synthetic amyloid B-protein. Judging
from these observations, the amount of the peptide
with amino acids 1-17 was not equally present in
these plaques. However, the amount of B amyloid
protein having amino acids 17-24 was approximately
the same in all the plaques as evidenced by equal
staining density. The availability of SV17-6E10 and
SCVAP-2F9 or SCVAP-4G8 makes it possible to reveal
for the first time that the plaques are composed of
more peptides with amino acid residues 17-24 and less
of the peptide having amino acid residues 1-17 in
some plaques.

The immunohistochemical staining procedure
used was as follows. The deparaffinized sections (6
um thickness) were washed in 0.05M Tris-based buffer
(TBS) (pH 7.6) and 0.15M NaCl (TBS) for 5-10 minutes
and immersed in undiluted 99% formic acid (Sigma
Chemical Co., St. Louis, MO) for 3-4 hours. They
were then washed with tap water (3-4 times) and then
washed with TBS (5-6 times). They were further
immersed in 0.3% hydrogen peroxide for 20 minutes and
washed with TBS (5-6 times). After treatment with
normal horse serum (Vector stain Elite ABC Kit,
anti-mouse IgG made in horse) for 30 minutes, sections
were incubated with primary mouse monoclonal antibody
(SCVAP-2F9, SCVAP-4G8 or SV17-6E10) to amyloid
B-protein (diluted 1:50-4,000) overnight in a moist
chamber at room temperature. All slides were washed
with 0.05 M TBS after each incubation. Secondary
antibody (biotinylated anti-mouse IgG, Vector
Laboratories, Inc., Burlingame, CA) was applied for
30 minutes. Avidin-biotinylated peroxidase
(Vector-Stain Elite ABC Kit) was used for 45
minutes. They were then stained with 3,3'
-diaminobenzidine tetrahydrochloride (DAB) for 5-10

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minutes.   Sections were lightly stained with
Hematoxylin (RICCA Chemical Co., Arlington, TX) for
30 seconds, dehydrated and covered with permount and
cover slips.

Example 3
Fab Fragments Of Monoclonal Antibody SV17-6E10
Fab fragments of monoclonal antibody
SV17-6E10 were prepared and tested as follows.
Immunoglobulin IgG purified by protein A column was
concentrated to 20 mg/ml in 20 mM phosphate/10 mM
EDTA. A portion (0.5 ml) was mixed with digestion
buffer (0.5 ml; 42 mg cysteine - HCl in 12 ml
phosphate buffer; pH 7.0). Then, a portion (0.5 ml)
of 50% slurry of immobilized papain, which had been
rinsed twice with digestion buffer, was incubated (5
hours at 37°C) with constant shaking. The
solubilized Fab and Fc fragments and undigested IgG
from the immobilized papain gel were recovered. The
recovered supernatant was applied to a protein A
column which had been equilibrated with binding
buffer. Eluate contained Fab fragments.

The Fab fragments were dialyzed against PBS
and used for immunostaining of brain sections. When
avidin/biotin complex staining method or FITC
immunofluorescent staining procedures were used, the
Fab fragments of SV17-6E10 selectively stained only
amyloid plaques.

Example 4
Double Antibody Sandwich Immunoassay
In the experiments reported by this example,
B-amyloid protein was detected using the double
antibody sandwich procedure described in Kim, et al.,
J. Clin. Microbiol., 18:331 (1983).

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Falcon ELISA plates with 96 wells were coated with purified SV17-6E10 monoclonal antibody (50 ul; 5 ug/ml in carbonate buffer; pH 9.6) overnight at 4°C. After the plates were washed 10 times with distilled water, they were coated with PBS containing 10% goat serum, 3% bovine serum albumin, and 1% Triton X-100 (PBSTGB) for one hour at room temperature; washed 10x again with distilled water; were then coated with 50 ul of the 24 amino acid residue synthetic B-protein (referred to as "SCVAP2"; sequence = DAEFRHDSGYEVHHQKLFAEDV); diluted in PBSTGB; and were thereafter incubated for 2 hours at room temperature. Note that amino acid residue 11 is glutamine here.

After the plates were washed, the optimum dilution of horseradish peroxidase conjugated monoclonal antibody 4G8 (4G8-HRP) at 1/1,600 dilution was added and plates were incubated an additional 2 hours at room temperature. The plates were then washed for 20 minutes with PBST and a final wash with distilled water, and orthophenylene diamine (OPD) substrate solution (100 ul) was added. After 10 minutes, 75 ul of 1 M H_2SO_4 was added and the color change was determined with a Dynatech Microelisa Auto Reader MC640 with a 490 nm filter. A well that received only PBSTGB was used as a negative control. The cutoff point between positive and negative was the average optical density value of the negative controls plus 2 standard deviations. As reported below, SV17-6E10:4G8HRP refers to the reagent wherein SV17-6E10 is used as the capture antibody and 4G8HRP is the detection antibody. Alternatively, in the other embodiment reported, SCVAP-4G8 is the capture antibody and SV17-6E10HRP is the detecting antibody. The results are reported in Table 1.

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Table 1

Double Antibody ELISA Test

SCVAP2 (picog/ml)	6E10:4G8HRP	4G8:6E10HRP
	Relative OD Reading	
1. 500,000	658	1533
2. 250,000	662	1365
3. 125,000	786	1284
4. 62,500	694	1268
5. 31,250	756	1275
6. 15,625	610	681
7. 7,812.50	400	553
8. 3,906.25	222	274
9. 1,953.12	174	173
10. 976.56	125	82
11. 488.28	94	40
12. 244.14	9	23
13. 122.07	21	6
14. 61.04	28	9
15. 30.52	19	1
16. 15.25	31	7
17. 7.63	49	27
18. 3.81	43	35
19. 1.91	52	8
20. 0.95	51	0

6E10:4G8HRP ELISA Sensitivity 4G8:6E10HRP ELISA Sensitivity

Mean = 33.7   Mean = 11

Std. Dev. = 15.7   Std. Dev. = 13

Mean + Two Std. Dev. = 65.1   Mean + Two Std. Dev. = 37.0

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Positive results were defined as the highest
dilution of B-protein giving an optical density value
higher than the cut-off optical density value. Both
assay systems are capable of detecting as little as
488.28 picograms of B-amyloid synthetic peptide per

5   ml or 24.4 picogram of B-amyloid synthetic peptide
present in 50 ul of sample. Therefore, as long as
6E10 and 4G8 were used in combination of either
detecting or capture antibody the double antibody
sandwich ELISA was a very sensitive test. This

10   successful double antibody sandwich EL1SA system for
detecting B-protein has never been reported before.

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What Is Claimed Is:

1.   Monoclonal antibody SV17-6E10 and antigen -
binding fragments thereof.

2.   The monoclonal antibody or binding fragment of
claim 1 which is specifically reactive with a
cerebrovascular amyloid protein whose elevated levels
are characteristic of Alzheimer's disease.

3.   The monoclonal antibody or binding fragment of
claim 1 which is of subclass IgG1.

4.   The monoclonal antibody or binding fragment of
claim 1 which is specifically bindable with a peptide
comprising the sequence asp - ala - glu - phe - arg
- his - asp - ser - gly - tyr - gln - val - his - his
- gln - lys - leu.

5.   The monoclonal antibody or binding fragment of
claim 1 which is produced by a hybridoma formed by
fusion of cells from a mouse myeloma line and
antibody-producing cells from a mouse previously
immunized with a peptide whose concentration level
is elevated in individuals having Down's syndrome or
Alzheimer's disease as compared to individuals of
substantially the same age who are not so-afflicted
and which does not react with other peptides of human
origin.

6.   The monoclonal antibody or binding fragment of
claim 1 which is produced by a hybridoma formed by
fusion of cells from a mouse myeloma line and
antibody-producing cells from a mouse previously
immunized with a cerebrovascular amyloid protein
whose elevated levels are characteristic of
Alzheimer's disease or Down's syndrome.

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7. The monoclonal antibody or binding fragment of
claim 1 which is produced by a hybridoma formed by
fusion of cells from a mouse NSO myeloma line and
antibody-producing cells from a mouse previously
immunized with a peptide whose concentration level is
elevated in individuals having Down's syndrome or
Alzheimer's disease as compared to individuals of
substantially the same age who are not so-afflicted,
and which monoclonal antibody or binding fragment
thereof does not react with other peptides of human
origin.

8. The monoclonal antibody or binding fragment of
claim 1 which is produced by a hybridoma formed by
fusion of cells from a mouse NSO myeloma line and
antibody-producing cells from a mouse previously
immunized with a cerebrovascular amyloid protein
whose elevated levels are characteristic of
Alzheimer's disease or Down's syndrome.

9. A monoclonal antibody which is produced by a
hybridoma formed by fusion of cells from a mouse NSO
myeloma line and antibody-producing cells from a
mouse previously immunized with a peptide comprising
the sequence asp – ala – glu – phe – arg – his – asp
– ser – gly – tyr – gln – val – his – his – gln – lys
– leu or an antigen – binding fragment of said
monoclonal antibody.

10. Monoclonal antibody SV17-6E10 or an antigen –
binding fragment of said monoclonal antibody derived
from hybridoma cells formed from NSO myeloma cells
and spleen cells of a Balb/CJ mouse immunized with a
peptide having the sequence asp – ala – glu – phe –
arg – his – asp – ser – gly – tyr – gln – val – his –
his – gln – lys – leu which is conjugated directly or

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through a linkage group to keyhole - limpet
hemocyanin.

11. A hybridoma cell line capable of producing the
monoclonal antibody of claim 1.

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12. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a myeloma line and
antibody-producing cells previously immunized with a
peptide whose concentration level is elevated in
individuals having Down's syndrome or Alzheimer's

10   disease as compared to individuals of substantially
the same age who are not so-afflicted.

13. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a mouse myeloma line

15   and antibody-producing cells from a mouse previously
immunized with a peptide whose concentration level is
elevated in individuals having Down's syndrome or
Alzheimer's disease as compared to individuals of
substantially the same age who are not so-afflicted.

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14. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a mouse NSO myeloma
line and antibody-producing cells from a mouse
previously immunized with a peptide whose

25   concentration level is elevated in individuals having
Down's syndrome or Alzheimer's disease as compared to
individuals of substantially the same age who are not
so-afflicted.

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15. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a mouse NSO myeloma
line and antibody-producing cells from a mouse
previously immunized with a peptide comprising the
.sequence asp - ala - glu - phe - arg - his - asp -

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ser - gly - tyr - gln - val - his - his - gln - lys -
leu.

16. A composition for quantitatively determining a
peptide whose elevated levels are characteristic of
Alzheimer's disease or Down's syndrome in an
individual, which composition comprises the
monoclonal antibody or specific - binding fragment of
claim 1 and a detectable moiety which is directly or
indirectly associated therewith.

17. The composition of claim 16 wherein said
monoclonal antibody or specific - binding fragment is
conjugated to an enzyme and said detectable moiety
comprises a chromogenic redox substrate for said
enzyme.

18. The composition of claim 16 wherein said
monoclonal antibody or specific - binding fragment is
specifically bindable with a substance attached to
said detectable moiety.

19. The composition of claim 18 wherein the
substance attached to said detectable moiety is an
antibody specifically bindable with an immunoglobulin.

20. The composition of claim 16 wherein said
monoclonal antibody is conjugated to one partner of a
specific binding pair and which further comprises the
other partner of said specific binding pair
conjugated to said detectable moiety or a substance
capable of rendering said moiety detectable.

21. The composition of claim 20 wherein one partner
of said specific binding pair is selected from the
group of biotin and its binding analogs and the other

partner of said binding pair is selected from the
group of avidin and its binding analogs.

22. The composition of either of claims 20 or 21
wherein said detectable moiety is a chromophore,
fluorophore or luminophore and said substance capable
of rendering said moiety detectable is an energy
donor or catalyst therefor.

23. A method for quantitatively determining a
peptide whose elevated levels are characteristic of
Alzheimer's disease or Down's syndrome in an
individual, which method comprises contacting a sample
from said individual with the composition of any of
claims 16-20 and quantitatively observing any
detectable response.

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FIG. 1 (a)

FIG. 1 (b)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02003

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12N 5/20; C07K 15/28; G01N 33/533,534,535
U.S. CL: 530/387, 388; 435/240.27, 7, 188

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System ¹	Classification Symbols
U.S.	530/387, 388; 435/7, 188, 240.27; 935/104,108,110

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

Databases: STN International Online (File CA, 1967-1990-File Biosis, 1969-1990) Automated Patent System (File USPAT, 1975-1990). For: monoclonal antibody, amyloid, cerebrovascular, protein/polypeptide, and Alzheimers

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category * ¹	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	US, A, 4,666,829 (GLENNER ET AL.) 19 May 1987	1-15
Y	see entire document.	16-23
Y	Robakis et al., "Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neurity plaque amyloid peptides", Proc. Natl. Acad. Sci vol. 84: 4190-4194, Published June 1987. see especially p. 4192, Fig 2.	1-23
Y	Selkoe et al., "Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease" Science 235: 873-877, Published 20 February 1987. see especially page 874, column 1.	1-23
Y	Glenner et al., "Alzheimers disease:Initial report of the purification and characterization of a novel cerebrovascular amyloid protein" Biochem. Biophys. Res. Comm. 120: No.3, 885-890. Published 16 May 1984. see especially page 889.	1-23
Y	Glenner et al., "Alzheimer's disease and Down's Syndrome: sharing of a unique cerebrovascular amyloid fibril protein" Biochem. Biophys. Res. Comm. 122: 1131-1135. Published 16 August 1984. see especially pages 1131 and 1133, Table 1.	1-23

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure. use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²
25 JUNE 1990	27 AUG 1990

International Searching Authority ¹	Signature of Authorized Officer ²⁰
ISA/US	PAULA HUTZELL

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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Kang et al., "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor" _Nature_, vol. 325: 733-736 Published 19 February 1989. see especially page 735.	1-23
Y	Wong et al., "Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related" _Proc. Natl. Acad. Sci. USA_ 82: pages 8729-8732. Published December 1985. see especially page 8729.	1-23
Y	Goding, "Monoclonal antibodies: Principles and Practice" Published 1983 by Academic Press, Inc. (LONDON), see pages 56-97 and pages 209-249, especially pages 230-235.	1-23

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers ..... because they relate to subject matter [1] not required to be searched by this Authority, namely:

2.☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3.☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

1.☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the International application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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